Inhibition by Mercuric Chloride of Aquaporin-1 in the Parietal Sheep Peritoneum: An Electrophysiologic Study

Vassilios Liakopoulos,1 Sotirios Zarogiannis,2 Chrysa Hatzoglou,2 Panagioto Kourtì,1 Antigoni Poultsidi,1 Theodoros Eleftheriadis,1 Konstantinos Gourgoulianis,3 Pachalis-Adam Molyvdas,2 Ioannis Stefanidis1

The peritoneal mesothelium is a barrier to ion transport in peritoneal dialysis. In this study, we used Ussing-chamber experiments to investigate the effect of HgCl2, an aquaporin-1 inhibitor, on the transmesothelial electrical resistance (RTM) of isolated sheep parietal peritoneum.

Peritoneal samples from the diaphragm of adult sheep were isolated immediately after the death of the animal and were transferred within 30 minutes to the laboratory in a cooled Krebs–Ringer bicarbonate solution (4°C, pH 7.5) bubbled with 95% O2/5% CO2. A planar sheet of the parietal peritoneum was mounted in an Ussing-type chamber and HgCl2 (10^-4 mol/L) was added apically or basolaterally. The RTM was measured before and serially after the addition of HgCl2. The entire experimental apparatus was held at 37°C, because active ion transport is temperature-dependent. The results presented are the mean ± standard error of 12 experiments.

The control RTM (that is, before the addition of HgCl2) was 19.3 ± 0.38 Ω•cm2. Addition of HgCl2 apically induced a decrease in the RTM to 16.25 ± 0.86 Ω•cm2 within 1 minute. When added basolaterally, HgCl2 action was similar, with a rapid reduction in the RTM to 18.1 ± 0.51 Ω•cm2 (p < 0.05).

A clear association between the RTM and the active transmesothelial ion transport was shown in previous studies. In the present study, rapid action of HgCl2 on the permeability of the parietal peritoneum was observed, resulting in a reduction in the RTM. Taken together, these findings indicate that inhibition of aquaporin-1 alters the ionic permeability of the parietal peritoneal membrane.

Key words
Peritoneum, aquaporin-1, transmesothelial resistance, Ussing chamber

Introduction
Peritoneal dialysis (PD) is one of the major treatments for end-stage renal disease. The longevity of PD therapy depends on maintenance of adequate ultrafiltration (UF). One of the major problems associated with PD is UF failure, which can affect up to 50% of PD patients treated for more than 6 years. It has been proved that peritoneal permeability for small solutes increases with time on PD, eventually leading to UF failure and dropout from PD (1,2).

The peritoneal mesothelium is one of the barriers to water and ion transport from the peritoneal cavity to the peritoneal capillary bed (3). Solute and water transport across the peritoneal membrane during PD is best described by the three-pore model (4). That model suggests that most water transport is attributable to the ultrasmall pores of the peritoneal membrane. Previous studies (5) showed that the molecular correlate of the ultrasmall pores is aquaporin-1 (AQP-1). Furthermore, in knockout mice lacking the AQP-1 gene, a 60% reduction of the osmotically induced water transport across the peritoneal membrane was observed (6).

The ubiquitous membrane channel protein AQP-1 is abundantly present in the mesothelial cells of the peritoneum (7). It forms homotetramers in cell membranes, each monomer forming a functionally independent water pore that does not conduct protons, ions, or other charged solutes. A fifth pore is formed in the center of the tetramer. The passive transport of water across cell membranes remains the major physiologic function established for AQP-1. However, recent studies have indicated that the central pore of the AQP-1 tetramers may also conduct ions (8,9).
Several studies performed in Ussing chambers have showed a clear association between transmesothelial electrical resistance ($R_{TM}$) and the transcellular active-ion transport in serosal membranes such as the peritoneum (10–12), the pleura (13–17), and the pericardium (16). In these studies, the permeability alterations of the concrete membrane were investigated in relation to the action of substances such as sex hormones, channel blockers, NO inhibitors, catecholamines, and opioids.

In the present study, we used Ussing chamber experiments to examine the effect of mercuric chloride on the electrophysiologic properties of isolated sheep parietal peritoneum. Mercuric chloride is a potent, widely used inhibitor of AQP-1 (18). To our knowledge, the relationship between in vitro inhibition of AQP-1 and transmesothelial ionic permeability, as measured by $R_{TM}$, has never been previously investigated in the peritoneum.

**Materials and methods**

Intact sheets of sheep parietal peritoneum were obtained from adult male and female animals. The samples were collected from a slaughterhouse immediately after the animals were killed. (Time of warm ischemia was close to 0 minutes.) Samples were transferred within 30 minutes to the laboratory in an oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C. (Time of cold ischemia was less than 30 minutes.) The peritoneal tissue was immediately placed into a KRB solution bubbled with 95% O$_2$/5% CO$_2$, and the pH was adjusted to 7.4. The solution contained 117.5 mmol/L NaCl, 1.15 mmol/L NaH$_2$PO$_4$, 24.99 mmol/L NaHCO$_3$, 5.65 mmol/L KCl, 1.18 mmol/L MgSO$_4$, 2.52 mmol/L CaCl$_2$, and 5.55 mmol/L glucose. Pieces of the parietal peritoneum were isolated from the diaphragmatic peritoneum and were visually examined for holes and adherent tissue. Precautions were taken to avoid touching the surface. Additionally, after the experiments ended, several peritoneal samples were histologically examined to ensure lack of perforations and holes.

Specimens of parietal peritoneum were carefully mounted in the Ussing chambers (Dipl.-Ing. K. Mussler Scientific Instruments, Aachen, Germany), which had an opening surface area of 1 cm$^2$ (12). Tissues were bathed on each side of the membrane with 4 mL KRB solution, continuously oxygenated with 95% O$_2$/5% CO$_2$ and circulated by gas lift. Two pairs of Ag/AgCl electrodes monitored the transmesothelial potential difference (TPD) in millivolts and the transmesothelial resistance ($R_{TM}$) in ohms per square centimeter under open-circuit conditions. The two parameters TPD and $R_{TM}$ were measured every 6 seconds under current clamp conditions. Experiments were conducted simultaneously in three computer-controlled (clamp software version 2.14) chambers (12). Transmesothelial electrical parameters were measured in the basal state (that is, after an equilibrium time of 30–40 minutes), and during incubations with HgCl$_2$ apically or basolaterally. After the addition of HgCl$_2$ 10$^{-4}$ mol/L, maximal changes in the $R_{TM}$ were expressed as differences from baseline ($\Delta R_{TM}$). Because active transport of ions is influenced by temperature, the experimental apparatus was held at 37°C during measurements of transmesothelial electric parameters.

The experimental solution bathing the surface of the peritoneum that in vivo faces the peritoneal fluid is called the serosal solution; the solution bathing the surface that in vivo is exposed to the blood supply is called the mucosal solution. Here, the mesothelial cell membrane facing the fluid or the blood side is cited as the apical or basolateral membrane respectively.

During equal numbers of experiments ($n = 12$), KRB–HgCl$_2$ solution (10$^{-4}$ mol/L) was added to the serosal and to the mucosal solutions. All solutions were freshly prepared before each experiment, heated to 37°C and bubbled continuously with a 95% O$_2$/5% CO$_2$ gas mixture. The results presented in this study are the mean of 12 separate experiments.

After the addition of HgCl$_2$ in each bathing solution (mucosal or serosal), the voltage response to applied current pulses of 50 µA amplitude and 200 ms duration was measured within the first minute. The $R_{TM}$ was calculated, automatically deducting the resistance of the solution.

Statistical analysis was performed using SPSS for Windows (SPSS, Chicago, IL, U.S.A.). All data are expressed as mean ± standard error. The probability of error for comparison of the mean values was calculated using the t-test for paired data. Values of $p < 0.05$ were considered significant.

**Results**

The spontaneous electrical potential difference across the parietal peritoneum was not significantly different from zero (0.5 ± 0.21 mV). The $R_{TM}$ of the parietal
peritoneum in control conditions—that is, before the addition of HgCl₂, was \(19.3 \pm 0.38 \, \Omega \cdot \text{cm}^2\).

The \(R_{TM}\) decreased significantly to \(16.25 \pm 0.86 \, \Omega \cdot \text{cm}^2\) \((p < 0.05)\) within 1 minute after the addition of KRB–HgCl₂ solution \((10^{-4} \, \text{mol/L})\) apically. After addition of KRB–HgCl₂ solution basolaterally, the \(R_{TM}\) decreased significantly to \(18.1 \pm 0.51 \, \Omega \cdot \text{cm}^2\) \((p < 0.05)\) also within 1 minute (Figure 1).

The effect of HgCl₂ was more pronounced apically, and a statistically significant difference \((p < 0.05)\) was observed between the \(R_{TM}\) in the first minute after the addition of HgCl₂ apically and the \(R_{TM}\) after the addition of HgCl₂ basolaterally.

**Discussion**

In PD, AQP-1 provides a major route for osmotically driven water transport across the peritoneal barrier (6). Furthermore, as shown in a previous study, AQP-1 is recruited to the plasma membrane by hyperosmotic stimuli via a protein kinase A–dependent pathway in rat peritoneal mesothelial cells (19). However, in AQP-1 knockout mice, in the absence of osmotic stimuli, AQP-1 depletion did not particularly affect the fluid transport (6).

In the current study, we investigated the effect of AQP-1 inhibition on the electrophysiologic properties of sheep parietal peritoneum. In particular, we evaluated the transmesothelial potential difference and transmesothelial electrical resistance. In aqueous solutions, electrical currents are carried by ions. Therefore, the transmesothelial potential difference is an index of net ion transport (20). On the other hand, transmesothelial electrical resistance is a measure of mesothelial ionic permeability. Mercuric chloride was used as an inhibitor of AQP-1 channels.

Our findings show that, across the sheep parietal peritoneum, there is no measurable spontaneous potential difference \((0.5 \pm 0.21 \, \text{mV} \) is not significantly different from zero) and only a very low ohmic resistance \((R_{TM} \; 19.3 \pm 0.38 \, \Omega \cdot \text{cm}^2)\). These findings indicate that the peritoneal membrane has electrophysiologic properties similar to those of other “leaky” epithelial tissues such as the renal proximal tubule, rabbit gallbladder, and sheep pleura (13).

After the addition of HgCl₂, apically and basolaterally alike, the ohmic resistance of the sheep parietal peritoneum declined significantly. The HgCl₂ decreased the \(R_{TM}\) of the parietal peritoneum within 1 minute. The rapid commencement of the HgCl₂ effect suggests that its action is mediated by AQP-1 channel inhibition. Our findings also clearly indicate that inhibition of AQP-1 makes the peritoneal mesothelium more permeable to ionic currents. The decrease in the \(R_{TM}\) is greater when HgCl₂ is added apically. We hypothesize, therefore, that more AQP-1 channels may be present apically.

The physiologic basis of our observations cannot be attributed merely to inhibition of the water transport properties of AQP-1 after treatment with HgCl₂. On the contrary, our observations enhance the hypothesis previously suggested in electrophysiologic studies with lipid bilayers that AQP-1 possesses ionic transport properties in addition to specific water transport properties (8,9). Furthermore, as a possible explanation, an upregulation of other ionic transporters on the mesothelium after the inhibition of AQP-1 could be hypothesized.

**Conclusions**

Our results agree with previous studies that clearly showed the existence of AQP-1 on the mesothelial cells of the peritoneum. They further indicate that the inhibition of AQP-1 by HgCl₂ influences ionic transport properties of sheep parietal peritoneum.
References


Corresponding author:
Ioannis Stefanidis, MD, Associate Professor of Internal Medicine/Nephrology, University of Thessaly, School of Medicine, Papakyriazi 22, Larissa 41222 Greece.
E-mail: stefanid@med.uth.gr